

CLAIMS

What is claimed is:

- 5        1) A method for maintaining genetic lines in a non-human mammal comprising:  
          a. isolating oocytes from a donor female of said non-human mammal to  
          produce isolated oocytes;  
          b. reducing the circumferential thickness of the zona pellucida layer  
          surrounding said isolated oocytes without removing said zona pellucida  
          layer completely;  
          c. fertilizing said isolated oocytes in vitro with cryogenically preserved  
          sperm from the same species as said non-human mammal to produce at  
          least one fertilized embryo; and  
          d. transplanting said fertilized embryo to a recipient female of said non-  
          human mammal for implantation and placental development.
- 10        2) The method of Claim 1, wherein said method further comprises the step of  
          inducing said donor female to superovulate prior to said isolating.
- 15        20        3) The method of Claim 1, wherein said reducing comprises reducing the  
          circumferential thickness of the zona pellucida layer surrounding said isolated  
          oocytes by approximately one-half.
- 25        4) The method of Claim 1, wherein said reducing comprises treating said zona  
          pellucida layer with acid Tyrode's solution.
- 5) The method of Claim 1, wherein said reducing comprises treating said zona  
          pellucida layer with an acidified salt solution.

- 6) The method of Claim 5, wherein said acidified salt solution is chosen from the group consisting of acidified PBS, acidified HTF and acidified normal saline solution.
- 5 7) The method of Claim 1, wherein said reducing comprises treating said zona pellucida layer with an enzymatic agent.
- 10 8) The method of Claim 7, wherein said enzymatic agent is a proteinase which is capable of reducing said zona pellucida layer.
- 15 9) The method of Claim 8, wherein said proteinase is chosen from the group consisting of pronase, hyaluronidase and trypsin.
- 10) The method of Claim 1, wherein said method further comprises the step of cryogenically preserving said sperm with a solution consisting of raffinose and dehydrated milk prior to said fertilizing.
- 15 11) The method of Claim 1, wherein said method further comprises the step of culturing said fertilized embryo to at least the 2-cell embryo stage prior to said transplanting.
- 20 12) The method of Claim 1, wherein said method further comprises the step of culturing said fertilized embryo to at least the blastocyst stage prior to said transplanting.
- 25 13) The method of Claim 1, wherein said non-human mammal is a mouse.
- 14) A method for manipulating the zona pellucida of a non-human mammalian oocyte to increase the fertilization capability of cryopreserved sperm such that the circumferential thickness of said zona pellucida surrounding said
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mammalian oocyte is decreased while maintaining an intact zona pellucida layer, comprising:

- a. removing cumulus cells surrounding said oocyte; and
- b. treating said oocyte with an agent to reduce the circumferential thickness of said zona pellucida

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15) The method of Claim 14, wherein said circumferential thickness of said zona pellucida is reduced by approximately one-half.

10 16) The method of Claim 14, wherein said agent is acid Tyrode's solution.

17) The method of Claim 14, wherein said agent is an acidified salt solution.

15 18) The method of Claim 17, wherein said acidified salt solution is chosen from the group comprising acidified HTF, acidified PBS and acidified normal saline solution.

19) The method of Claim 14, wherein said agent is an enzymatic agent.

20 20) The method of Claim 19, wherein the enzymatic agent is a proteinase capable of reducing said circumferential thickness of the zona pellucida layer.

21) The method of Claim 20, wherein said proteinase is chosen from the group comprising pronase, hyaluronidase and trypsin.

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22) An isolated oocyte wherein the circumferential thickness of the zona pellucida layer surrounding said isolated oocyte is reduced.

- 23) The isolated oocyte of Claim 22, wherein said circumferential thickness of the zona pellucida layer surrounding said isolated oocyte is reduced by approximately one-half.
- 5 24) The isolated oocyte of Claim 22, wherein said isolated oocyte is treated with an acidified salt solution.
- 10 25) The isolated oocyte of Claim 22, wherein said acidified salt solution is acid Tyrode's solution.
- 26) The isolated oocyte of Claim 22, wherein said isolated oocyte is treated with an enzymatic agent.
- 15 27) The isolated oocyte of Claim 26, wherein said enzymatic agent is a proteinase capable of reducing said circumferential thickness of the zona pellucida layer.
- 28) The isolated oocyte of Claim 27, wherein said proteinase is chosen from the group comprising pronase, hyaluronidase and trypsin.